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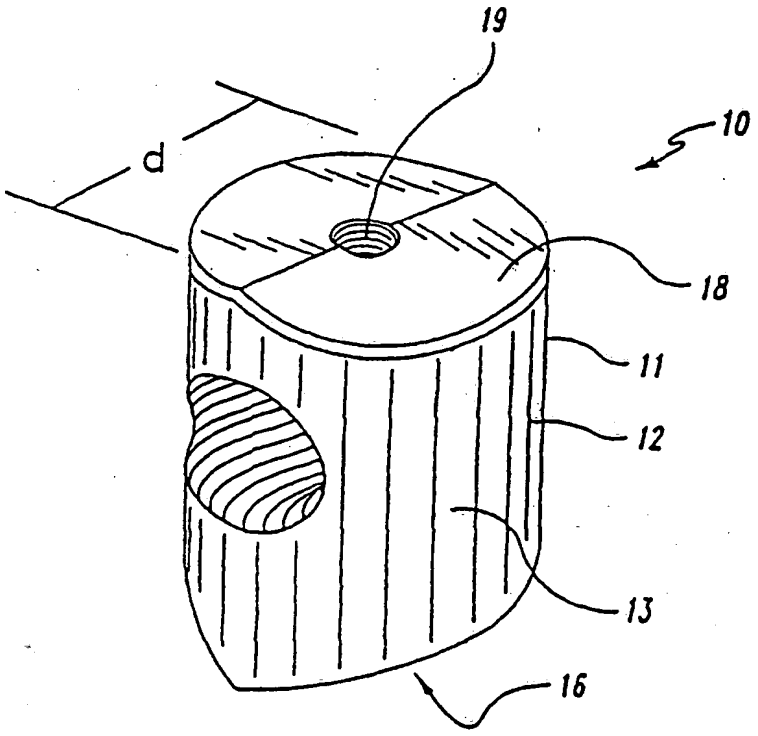

(57) Abstract: The present invention relates to reduced antigenicity tissue (RAT) implants, including bone implant materials comprising reduced antigenicity bone (RAB) and cartilage implant materials comprising reduced antigenicity cartilage (RAC) by virtue of having been treated to remove substantially all non-collagenous proteins from the bone or cartilage implant matrix. In specific applications of the invention the RAB and RAC implants are treated with osteogenic (osteoinductive or osteoconductive) or cartilage growth inducing compositions, including but not limited to bone morphogenic proteins, cartilage derived morphogenic proteins, growth factors, peptides, cells, natural or recombinant, expressing such proteins or growth factors, and expressible nucleic acids encoding such proteins, peptides, growth factors, or combinations thereof.

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TITLE OF THE INVENTION

REDUCED ANTIGENICITY TISSUE (RAT) IMPLANTS

FIELD OF THE INVENTION

The present invention relates to bone, cartilage and other tissue implant materials comprising reduced antigenicity bone (RAB), reduced antigenicity cartilage (RAC) or reduced antigenicity tissue (RAT) by virtue of having been treated to remove substantially all non-collagenous proteins from the bone, cartilage or other tissue implant matrix. In specific applications of the invention the RAB, RAC and RAT implants are treated with osteogenic (osteoinductive or osteoconductive) compositions, including but not limited to bone morphogenic proteins, cartilage derived morphogenic proteins, growth factors, cells, natural or recombinant, expressing such proteins or growth factors, and expressible nucleic acids encoding such proteins and growth factors.

BACKGROUND OF THE INVENTION

In the art of orthopedic medicine, there is frequently the need for implantation of materials in order to provide support or to replace damaged or diseased bone tissue. Classically, such implants have comprised titanium or other relatively inert metals, synthetic polymeric substances, and the like. Autologous bone, harvested from a first anatomical location, and reimplanted into a second anatomical location, has also been relied upon by surgeons. However, such methods, while effective at the second anatomical location, are less than ideal, due to morbidity at the first anatomical location. Use of allograft (from another individual of the same species) or xenograft (from another species) bone, cartilage or other material has gained increasing acceptance, as techniques for removal of potentially pathogenic organisms have become increasingly sophisticated and reliable. However, residual problems exist with induction of immune responses to antigenic proteins present in allograft and xenograft implants. Accordingly, there has been a long felt need for materials which may reliably be obtained in substantially unlimited quantities, such as xenograft bone (e.g. bovine, ovine, equine, porcine, canine,

etc.), suitable for implantation in various orthopedic applications, such as spinal fusion, and the like.

Spinal fusion is indicated to provide stabilization of the spinal column for painful spinal motion and disorders such as structural deformity, traumatic instability, degenerative instability, and post-resection iatrogenic instability. Fusion, or arthrodesis, is achieved by the formation of an osseous bridge between adjacent motion segments. This can be accomplished within the disc space, anteriorly between contiguous vertebral bodies or posteriorly between consecutive transverse processes, laminae or other posterior aspects of the vertebrae.

An osseous bridge, or fusion mass, is biologically produced by the body upon skeletal injury. This normal bone healing response is used by surgeons to induce fusion across abnormal spinal segments by recreating spinal injury conditions along the fusion site and then allowing the bone to heal. A successful fusion requires the presence of osteogenic or osteopotential cells, adequate blood supply, sufficient inflammatory response, and appropriate preparation of local bone. This biological environment is typically provided in a surgical setting by decortication, or removal of the outer, cortical bone to expose the vascular, cancellous bone, and the deposition of an adequate quantity of high quality graft material.

A fusion or arthrodesis procedure is often performed to treat an anomaly involving an intervertebral disc. Intervertebral discs, located between the endplates of adjacent vertebrae, stabilize the spine, distribute forces between vertebrae, and cushion vertebral bodies. A normal intervertebral disc includes a semi-gelatinous component, the nucleus pulposus, which is surrounded and confined by an outer, fibrous ring called the annulus fibrosis. In a healthy, undamaged spine, the annulus fibrosis prevents the nucleus pulposus from protruding outside the disc space. Spinal discs may be displaced or damaged due to trauma, disease or aging. Disruption of the annulus fibrosis allows the nucleus pulposus to protrude into the vertebral canal, a condition commonly referred to as a herniated or ruptured disc. The extruded nucleus pulposus may press on the spinal

nerve, which may result in nerve damage, pain, numbness, muscle weakness and paralysis. Intervertebral discs may also deteriorate due to the normal aging process or disease. As a disc dehydrates and hardens, the disc space height will be reduced leading to instability of the spine, decreased mobility and pain.

Sometimes the only relief from the symptoms of these conditions is a discectomy, or surgical removal of a portion or all of an intervertebral disc, followed by fusion of the adjacent vertebrae. The removal of the damaged or unhealthy disc will allow the disc space to collapse. Collapse of the disc space can cause instability of the spine, abnormal joint mechanics, premature development of arthritis or nerve damage, in addition to severe pain. Pain relief via discectomy and arthrodesis requires preservation of the disc space and eventual fusion of the affected motion segments. Bone grafts are often used to fill the intervertebral space to prevent disc space collapse and promote fusion of the adjacent vertebrae across the disc space. In early techniques, bone material was simply disposed between the adjacent vertebrae, typically at the posterior aspect of the vertebrae, and the spinal column was stabilized by way of a plate or rod spanning the affected vertebrae. Once fusion occurred the hardware used to maintain the stability of the segment became superfluous and was a permanent foreign body. Moreover, the surgical procedures necessary to implant a rod or plate to stabilize the level during fusion were frequently lengthy and involved. It was therefore determined that a more optimal solution to the stabilization of an excised disc space is to fuse the vertebrae between their respective end plates, preferably without the need for anterior or posterior plating.

There have been a number of attempts to develop an acceptable intra-discal implant that could be used to replace a damaged disc and maintain the stability of the disc interspace between the adjacent vertebrae, at least until complete arthrodesis is achieved. To be successful the implant must provide temporary support and allow bone ingrowth. Success of the discectomy and fusion procedure requires the development of a contiguous growth of bone to create a solid mass, because the implant may not withstand the cyclic compressive spinal loads for the life of the patient. Many attempts to restore the intervertebral disc space after removal of the disc have relied on metal devices. U.S.

Patent No. 4,878,915 to Brantigan teaches a solid metal plug. U.S. Patent Nos. 5,044,104; 5,026,373 and 4,961,740 to Ray; 5,015,247 to Michelson and U.S. Patent No. 4,820,305 to Harms et al., U.S. Patent No. 5,147,402 to Bohler et al. and 5,192,327 to Brantigan teach hollow metal cage structures. Unfortunately, due to the stiffness of the material, some metal implants may stress shield the bone graft, increasing the time required for fusion or causing the bone graft to resorb inside the cage. Subsidence, or sinking of the device into bone, may also occur when metal implants are implanted between vertebrae if fusion is delayed. Metal devices are also foreign bodies which can never be fully incorporated into the fusion mass.

Various bone grafts and bone graft substitutes have also been used to promote osteogenesis and to avoid the disadvantages of metal implants. Autograft is often preferred because it is osteogenic. Both allograft and autograft are biological materials which are replaced over time with the patient's own bone, via the process of creeping substitution. Unlike a metal implant, over time a bone graft may virtually disappear, while a metal implant persists long after its useful life. Stress shielding is avoided because bone grafts have a similar modulus of elasticity as compared with the surrounding bone. Commonly used implant materials have stiffness values far in excess of both cortical and cancellous bone. Titanium alloy has a stiffness value of 114 Gpa and 316L stainless steel has a stiffness of 193 Gpa. Cortical bone, on the other hand, has a stiffness value of about 17 Gpa. Moreover, bone as an implant also allows excellent postoperative imaging because it does not cause scattering like metallic implants on CT or MRI imaging.

Various implants have been constructed from bone or graft substitute materials to fill the intervertebral space after the removal of the disc. For example, the Cloward dowel is a circular graft made by drilling an allogeneic or autogeneic plug from the illium. Cloward dowels are bicortical, having porous cancellous bone between two cortical surfaces. Such dowels have relatively poor biomechanical properties, in particular a low compressive strength. Therefore, the Cloward dowel is not suitable as an intervertebral spacer without internal fixation due to the risk of collapsing prior to fusion under the

intense cyclic loads of the spine. Bone dowels having greater biomechanical properties have been produced and marketed by the Regeneration Technologies, Inc., (RTI), 1 Innovation Drive, Alachua, Florida 32615, and have been patented, see U.S. Patent No. 5,814,084. Unicortical dowels from allogeneic femoral or tibial condyles are available. RTI has also developed a diaphysial cortical dowel having superior mechanical properties, which forms the basis of the 5,814,084 patent (the '814 patent). This dowel also provides the further advantage of having a naturally preformed cavity formed by the existing meduallary canal of the donor long bone. The cavity can be packed with osteogenic materials such as bone or bioceramic.

Unfortunately, the use of bone grafts can present several disadvantages. Autograft is available in only limited quantities. The additional surgery also increases the risk of infection and blood loss and may reduce structural integrity at the donor site. Furthermore, some patients complain that the graft harvesting surgery causes more short-term and long-term pain than the fusion surgery. Allograft material, which is obtained from donors of the same species, is more readily obtained. However, allogeneic bone does not have the osteoinductive potential of autogenous bone and therefore may provide only temporary support. The slow rate of fusion using allografted bone can lead to collapse of the disc space before fusion is accomplished. Both allograft and autograft present additional difficulties. Graft alone may not provide the stability required to withstand spinal loads. Internal fixation can address this problem but presents its own disadvantages such as the need for more complex surgery as well as the disadvantages of metal fixation devices. Also, the surgeon is often required to repeatedly trim the graft material to obtain the correct size to fill and stabilize the disc space. This trial and error approach increases the length of time required for surgery. Furthermore, the graft material usually has a smooth surface which does not provide a good friction fit between the adjacent vertebrae. Slippage of the graft may cause neural and vascular injury, as well as collapse of the disc space. Even where slippage does not occur, micromotion at the graft/fusion-site interface may disrupt the healing process that is required for fusion. In addition, even allograft material may be available in limited supply. Accordingly, a method for use of xenograft material has long been needed.

Several attempts have been made to develop a bone graft substitute which avoids the disadvantages of metal implants and bone grafts while capturing advantages of both. For example Unilab, Inc. markets various spinal implants composed of hydroxyapatite and bovine collagen. In each case, developing an implant having the biomechanical properties of metal and the biological properties of bone without the disadvantages of either, has been extremely difficult or impossible.

These disadvantages have led to the investigation of bioactive substances that regulate the complex cascade of cellular events of bone repair. Such substances include bone morphogenetic proteins, for use as alternative or adjunctive graft materials. Bone morphogenetic proteins (BMPs), a class of osteoinductive factors from bone matrix, are capable of inducing bone formation when implanted in a fracture or surgical bone site. Recombinantly produced human bone morphogenetic protein-2 (rhBMP-2) has been demonstrated in several animal models to be effective in regenerating bone in skeletal defects. The use of such proteins has led to a need for appropriate carriers and fusion spacer designs.

Due to the need for safer bone graft materials, bone graft substitutes, such as bioceramics, have recently received considerable attention. The challenge has been to develop a bone graft substitute which avoids the disadvantages of metal implants and bone grafts while capturing the advantages of both. Calcium phosphate ceramics are biocompatible and do not present the infectious or immunological concerns of allograft materials. Ceramics may be prepared in any quantity, which is a great advantage over autograft and even allograft bone graft material. Furthermore, bioceramics are osteoconductive, stimulating osteogenesis in bony sites. Bioceramics provide a porous matrix which further encourages new bone growth. Unfortunately, ceramic implants typically lack the strength to support high spinal loads and therefore require separate fixation before the fusion.

Of the calcium phosphate ceramics, hydroxyapatite(HA) and tricalcium phosphate (TCP) ceramics have been most commonly used for bone grafting. Hydroxyapatite is chemically similar to inorganic bone substance and biocompatible with bone. However,

it is slowly degraded. ß-tricalcium phosphate is rapidly degraded in vivo and is too weak to provide support under the cyclic loads of the spine until fusion occurs. Thus, developing an implant having the biomechanical properties of metal and the biological properties of bone without the disadvantages of either has been extremely difficult or impossible.

It recently became apparent that natural bone mineral is not actually as close to the chemistry and structure of hydroxyapatite as was previously believed. (Spector, 21 Clinics in Plastic Surgery 437-444, 1994, the complete text of which is herein incorporated by reference.) Natural bone mineral contains carbonate ions, magnesium, sodium, hydrogenophosphate ions and trace elements. Bone mineral also has a different crystalline structure than HA. Other details of bone chemistry are disclosed in U.S. Patent No. 4,882,149 to Spector. Mimicking the chemistry and microstructure of bone is important to obtain a beneficial modulus of elasticity and resorbption rate.

Several attempts have been made to make materials which are closer to the microstructure of bone. Some disclose removing organic material from bone to yield bone mineral. Some of the materials are used as drug carriers as disclosed in, for example, U.S. Patent No. 5,417,975. U.S. Patent No. 4,882,149 to Spector describes a bone mineral material which is free from fat and bone proteins. The result is a powdery, brittle radiopaque material which can be used to deliver bone growth proteins. The Spector mineral is thought to be closer to natural bone mineral than synthetic calcium phosphate ceramics, but it does not have characteristics which allow it to be shaped into formed objects. U.S. Patent Nos. 4,314,380 to Miyata et al. And 5,573,771 disclose adding collagen or gelatin to bone mineral. However, it is unclear how close these materials are to the natural structure of bone, because the crystalline structure is disrupted when all of the proteins are removed from the treated bone. Urist et al. (110 Arch Surg. 416, 1975) discloses a chemosterilized, antigen-extracted, autodigested, alloimplant which is thought to preserve the morphogenetic potential of the material. McKay, WO98/56433, published 17 December 1998, purported to disclose a bone graft composite and spacers comprising bone stated to have "been processed to remove associated non-

collagenous bone proteins", followed by combination through soaking with a bone growth factor. However, in reviewing the methods disclosed therein for removal of non-collagenous proteins from bone, it is apparent that removal of non-collagenous protein, and preservation of the collagenous structure of bone, would not be effectively accomplished. The "deactivation" process consisted of: chemical and enzymatic treatment to dissolve and remove all cellular and non-collagenous proteinaceous material. How the collagenous material is preserved is not disclosed. The thus treated material was then, purportedly, treated by soaking in isopronanol, peroxide, and SDS, followed by a rinse in water and gamma irradiation. None of these materials are thought to yield a non-collagenous-protein-free bone implant material comprising natural bone collagen and mineral which is identical to natural bone. Thus, a need has remained for fusion spacers which stimulate bone ingrowth and avoid the disadvantages of metal implants and known bone implants, yet provide sufficient strength to support the vertebral column until the adjacent vertebrae are fused. A need has also remained for bone graft materials which provide the osteogenic potential and low risk of infectious or immunogenic complications of autograft without the disadvantages of autograft.

SUMMARY OF THE INVENTION

In accordance with one aspect of the invention, bone graft compositions, vertebral spacers, and various other bone implants composed of bone graft compositions are provided. In one aspect, the invention provides reduced antigenicity bone (RAB) compositions, alone or in combination with a bone growth factor, bone morphogenic protein, cartilage derived growth factors, cells expressing such factors, or nucleic acids actively encoding such factors. In another aspect of this invention, reduced antigenicity cartilage (RAC) compositions, or reduced antigenicity tissue (RAT) compositions are prepared and used to replace damaged, diseased or otherwise compromised tissues, either in the spine or in any number of other biological locations.

One object of the invention is to provide a bone graft implant having substantially natural mineral structure, reduced antigenicity (reduced immunogenicity), safety and osteoinductive potential of autograft.

Another object of the invention is to provide a cartilage graft implant having substantially natural mineral structure, reduced antigenicity (reduced immunogenicity), safety and osteoinductive potential of autograft.

Another object of the invention is to provide a tissue graft implant having substantially natural structure, reduced antigenicity (reduced immunogenicity), safety and renewed tissue growth and induction potential.

Another object of the invention is to provide spacers for engagement between vertebrae which restore the intervertebral disc space and which support the vertebral column while encouraging bone ingrowth and avoiding stress shielding.

Another object of the invention is to provide pins, suture anchors, interference screws, demineralized bone implants, including but not limited to ligaments, oral maxilofacial plates, dowels, posterior lumbar interbody fusion implants, trauma screws and plates, pericardium (for dura, plura, shoulder patch and perioligaments), wedges, chips and pastes comprising reduced antigenicity bone, cartilage or other tissues, alone or in combination with growth factors, or nucleic acids encoding growth factors, including but not limited to bone morphogenetic proteins, cartilage derived morphogenetic proteins, tissue growth factor (beta1 and the like).

One benefit of the present invention is that it solves many of the problems associated with the use of bone and other graft materials, either from allograft or xenograft materials. The antigen removal process disclosed herein removes immunogenic and potentially disease causing agents while retaining the natural microstructure of bone and other tissues described herein. This feature allows the use of allograft or xenograft, which is available in virtually unlimited supply. Fortifying the graft with a bone growth factor, cells

expressing bone growth factors, or nucleic acids which actively encode bone growth factors or osteogenic proteins, makes the graft osteoinductive, thereby making the pain and risk of harvesting autograft unnecessary. An additional benefit is that the invention provides a stable scaffold for bone, cartilage or other tissue ingrowth as the process of fusion or new cartilage or tissue generation occurs.

A further object and another benefit of this invention is that it allows the use of bone grafts without the need for metal cages or internal fixation, due to the increased speed of fusion.

Other objects and further benefits of the present invention will become apparent to persons of ordinary skill in the art from the following written description and accompanying Figures.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a top perspective view of a bone dowel implant according to US Patent No. 5,814,084, treated according to the method of the present invention to remove non-collagen protein, and optionally treated with BMP, cells expressing growth factors, or nucleic acids encoding growth factors.

FIG. 2 shows bilateral dowel placement between L5 and the sacrum, using a RAB dowel such as that shown in figure 1.

FIG. 3 is a perspective view of a cortical RAB dowel such as that shown in figure 1, having a chamber and a threaded external feature.

FIG. 4 is a side perspective view of a RAB dowel according to this invention.

FIG. 5 is a cross-section of a RAB dowel of this invention.

FIG. 6 is a side elevational view of the RAB dowel shown in FIG. 5.

FIG. 7 is a RAB cortical ring packed with an osteogenic material.

FIG. 8 is a representation of a RAB cortical ring embodiment provided by this invention.

FIG. 9 is another embodiment of a RAB cortical ring provided by this invention.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

For the purposes of promoting an understanding of the principles of the invention, reference will now be made to the embodiments illustrated in the drawings and specific language will be used to describe the same. It will nevertheless be understood that no limitation of the scope of the invention is thereby intended, such alterations and further modifications in the illustrated spacers, and such further applications of the principles of the invention as illustrated therein being contemplated as would normally occur to one skilled in the art to which the invention relates.

The present invention provides bone graft compositions, spacers and surgical procedures. The bone graft compositions include reduced antigenicity bone (RAB) grafts, optionally in combination with an osteogenic material, such as a bone morphogenic protein (BMP), cartilage derived morphogenic protein, growth factors, cells expressing such proteins, peptides (e.g. p15) or nucleic acids actively encoding such growth factors, peptides or proteins. As is now known in the art, delivery of nucleic acids encoding desirable gene products results in uptake of such nucleic acids and expression of the encoded proteins. The nucleic acids may be so-called naked DNA or RNA, comprising appropriate transcription and translation start and stop signals, as are known in the art. The nucleic acid may also comprise viral replication signals.

This invention also provides pins, suture anchors, interference screws, demineralized bone implants, including but not limited to ligaments, oral maxilofacial plates, dowels,

posterior lumbar interbody fusion implants, trauma screws and plates, pericardium (for dura, plura, shoulder patch and perioligaments), wedges, chips and pastes comprising reduced antigenicity bone, cartilage or other tissues, alone or in combination with growth factors, or nucleic acids encoding growth factors, including but not limited to bone morphogenetic proteins, cartilage derived morphogenetic proteins, tissue growth factor (beta1 and the like). Thus, while emphasis may be placed herein on RAB implants for spinal fusions, those skilled in the art will appreciate, based on the instant disclosure, that RAC and RAT implants for a wide variety of orthopedic and non-orthopedic applications may benefit by treating such tissues to reduce antigenicity, and optionally treating such tissues with appropriate growth factors, cells, nucleic acids and the like.

The bone grafts according to this invention are treated according to the method disclosed herein to remove all of the cellular material, fat and non-collagenous protein that is otherwise associated with bone graft compositions. In preferred embodiments, free collagen is also removed, leaving structural or bound collagen which is associated with bone mineral to form the trabecular struts of bone.

Although the RAB graft of this invention is depleted of non-collagenous proteins and non-structural collagens and is defatted, it still contains the natural crystalline structure of bone. Therefore, the RAB bone graft compositions of this invention have the natural microstructure of bone without the risk of disease transmission or significant immunogenicity or antigenicity.

The natural crystalline structure of bone is maintained by the presence of structural collagen in association with the natural bone minerals. This yields a bone graft material with preferred physical and biological characteristics, including the ability to deliver bone growth factors and osteogenic proteins, cells, and nucleic acids, without attendant immunogenicity or antigenicity.

The presence of structural collagen and the natural mineral structure of bone results in an elasticity and radioopacity which is identical or nearly identical to bone. The material

has sufficient resilience and elasticity to retain a formed body and yet remains rigid enough to maintain an open space between bone portions to result in a fusion mass. Other allograft materials such as demineralized bone matrix do not have the optimal physical properties to accomplish this without the assistance of a support.

When the RAB graft materials of this invention are combined with an osteogenic factor such as bone morphogenetic protein, growth factor, cells expressing BMPs or growth factors, TGF-beta, TGF-beta superfamily members, FGF, PDGF, P15, or nucleic acids encoding BMPs, CDMPs (cartilage derived morphogenic proteins), TGF-beta, TGF-beta superfamily members, FGF, PDGF, P15, other growth factors, or nucleic acids encoding such factors, the composite is an ideal bone graft substitute. The composite has the natural calcium phosphate structure of bone. This facilitates incorporation and substitution of the graft material, giving the composites a desirable resorbption rate of a few months. This compares favorably to the resorbption rates of known materials which are typically either too fast, slow or unpredictable. For example, allograft typically is resorbed within 12-60 months but may, on the other hand, resorb too quickly before fusion can occur due to an immunogenic response by the patient.

The combination of BMP and other osteogenic factors with the RAB graft according to this invention provides the osteoinductive potential of autograft without the need for a harvesting surgical procedure at a secondary location, where morbidity may occur. The osteoinductive composites of this invention enhance bone growth into and incorporation of the graft, resulting in fusion more quickly than would occur using RAB graft material alone. Allograft alone typically requires many months to incorporate and sometimes is never fully incorporated, but is merely encased within the patient's bone. The quicker fusion, occurring within about five months, provided by this invention compensates for the less desirable biomechanical properties of graft and makes the use of internal fixation and metal interbody fusion devices unnecessary. The spacers of this invention are not required to support the cyclic loads of the spine for very long because of the quick fusion rates which reduce the biomechanical demands on the spacer. However, when required,

the compositions of this invention may be used with internal fixation devices or may be reinforced as needed, see WO98/56319, hereby incorporated by reference.

A further advantage provided by this invention is that, because the bone graft material of this invention has been treated to remove essentially all non-collagenous proteins and non-structural collagens, the graft may be autogeneic, allogeneic or xenogeneic. The components of bone which could cause disease or prompt the patient's body to reject the graft are removed by the treatment process disclosed herein. Xenogenic bone, such as bovine, ovine, porcine, canine, equine or other bone, is available in virtually unlimited supply. Several osteogenic factors are also available in unlimited supply thanks to recombinant DNA technology. Therefore, the present invention solves all of the problems associated with autograft, allograft and xenograft, including supply, immunogenicity, disease transmission and the need for surgical procedures at secondary sites.

This invention provides the further advantage of exploiting the discovery that bone mineral is an excellent carrier for osteogenic factors, such as bone morphogenic proteins, CDMP, nucleic acids encoding such factors, peptides (e.g. p15) and cells expressing such factors. Hydroxyapatite, which is similar in chemical composition to the mineral in cortical bone, is an osteogenic factor-binding agent which controls the rate of delivery of certain proteins to the fusion site. Calcium phosphate compositions such as hydroxyapatite are thought to bind bone morphogenic proteins and prevent BMP from prematurely dissipating from the spacer before fusion can occur. It is further believed that retention of the BMP by the agent permits the protein to initiate the transformation of mesenchymal stem cells into bone producing cells or osteoblasts within the device at a rate that is conducive to complete and rapid bone formation and ultimately, fusion across the disc space. The RAB spacers of this invention have the advantage of including a load bearing member composed of bone which naturally binds and provides controlled delivery of osteogenic factors such as bone morphogenic proteins, without at the same time inducing undesirable immune responses in the recipient thereof. We have also unexpectedly noted an approximately twelve-fold greater response to BMP when

incorporated into a bone carrier matrix and implanted, as compared to a collagen sponge impregnated with BMP.

This invention also capitalizes on the discovery that cortical bone, like metal, can be conveniently machined into the various shapes disclosed herein. In some embodiments, the load bearing members define threads on an outer surface. Machined surfaces, such as threads, provide several advantages that were previously only available with metal implants. Threads allow better control of spacer insertion than can be obtained with a smooth surface. This allows the surgeon to more accurately position the spacer, which is extremely important around the critical neurological and vascular structures of the spinal column.

Threads and the like also provide increased surface area which facilitates the process of bone healing and creeping substitution for replacement of the donor bone material and fusion. These features also increase post-operative stability of the spacer by engaging the adjacent vertebral endplates and anchoring the spacer to prevent expulsion. This is a major advantage over smooth grafts. Surface features also stabilize the bone-spacer interface and reduce micromotion to facilitate incorporation and fusion.

The RAB graft compositions of this invention can be prepared according to methods disclosed herein. Bone of human or animal source is obtained according to known procedures. The bone is cleaned to remove tissue and blood and is then treated with agents to remove cellular material, fats, noncollagenous proteins, and non-structural collagens. Typical agents include alcohols and peroxides. In preferred embodiments, the bone material is also treated to remove free collagen, leaving only bound or structural collagen in association with bone minerals. This reduces immunogenicity/antigenicity, without compromising the structural integrity of the bone material. One preferred agent for removing free collagen and associated non-structural antigenic proteins and any remaining fat is a chaotropic agent, such as urea, guanidinium hydrochloride, Triton X-100, Tween, TNBP, or the like, in combination with alcohol and peroxide treatment. The RAB bone material is then preferably washed with sterile, deionized water and terminally

sterilized by suitable methods, including but not limited to gamma irradiation, vapor-phase peroxide treatment, and the like.

RAB allograft or xenograft bone dowels or other appropriately shaped implants can be packaged fresh frozen or freeze-dried, preferably freeze dried. Sterilization can be provided via aseptic processing or terminally sterilized by ETO, E-beam, or gamma irradiation preferably gamma irradiation. Gamma irradiation allows the procurement and processing of the allograft under less rigorous environmentally controlled conditions since terminal sterilization offers a significantly higher degree of sterility.

The RAB graft according to this invention is treated to remove all of the non-collagenous bone proteins leaving a non-immunogenic, disease-free, bone graft implant material having the natural mineral, microcrystalline structure of bone, with a consistency which retains desired forms. The composition of this invention is preferred because it has a microstructure which is the closest to natural bone of all of the known treated bone products. This bone product also has the radioopacity of natural bone and does not show the dense white image of the bone products of Spector and Geistlich. The product of this invention also provides superior resorbability, particularly when combined with an osteogenic protein, cell, nucleic acid or other osteogenic factor. Resorbption has been found to advantageously occur within several months as opposed to several years required for the Spector and Geistlich materials or the few weeks of the Urist product. When the material is combined with a bone growth factor, the resorbption time is ample for forming the bony bridge required for fusion and bone healing. The RAB material of this invention also has an elasticity similar to normal bone while the Spector and Geistlich materials have been found to be brittle and weak.

The RAB materials of this invention are preferably combined with an osteogenic composition or material containing a bone growth factor, proteins, peptides, or cells expressing such factors, or nucleic acids which actively encode such factors. An osteogenic material can be applied to the bone material by impregnating the graft with a solution including an osteogenic composition. The allograft or xenograft is allowed to

soak for sufficient time to allow the allograft or xenograft to absorb the protein, nucleic acid, or is cultured with appropriate cells which encode growth factors. Additional protein could be used with the allograft or xenograft produced according to the method of this invention by the incorporation of the protein in a delivery vehicle placed around or in the allograft. In some embodiments, an osteogenic composition can be packed into a chamber defined within a body of the material. The various osteogenic factors, growth factors, proteins, peptides or nucleic acids may be forced into the interstices of the bone or other reduced antigenicity implants under vacuum or pressure, or by oscillation between high and low pressure in an appropriate chamber or vessel. The composition may be applied by the surgeon during surgery or the spacer may be supplied with the composition preapplied. In such cases, the osteogenic composition may be stabilized for transport and storage such as by freeze-drying. The stabilized composition can be rehydrated and/or reactivated with a sterile fluid such as saline or water or with body fluids applied before or after implantation.

The term "osteogenic composition" as used herein means virtually any material that promotes bone growth or healing, including natural, synthetic and recombinant proteins, hormones and the like, cells expressing such factors, and nucleic acids actively encoding such factors. By "actively encoding" is meant the inclusion in a nucleic acid construct of all required signals, including transcriptional promoters and terminators, enhancers, and the like, as known in the art, in order to achieve efficient expression of encoded factors. The osteogenic compositions used in this invention preferably comprise an amount of such composition sufficient to stimulate or induce bone growth or healing of a substantially pure bone inductive factor such as a bone morphogenetic protein in a pharmaceutically acceptable carrier. The preferred osteoinductive factors include, but are not limited to, the recombinant human bone morphogenic proteins (rhBMPs), CDMPs, and nucleic acids encoding such factors, because they are available in unlimited supply and do not transmit infectious diseases. Most preferably, the bone morphogenetic protein is a rhBMP-2, rhBMP-4 or heterodimers thereof. The concentration of rhBMP-2 or other growth factors (e.g. TGF-β1, TGF-β2, p15, and the like, available from many commercial sources, including but not limited to R&D Systems, Minneapolis, MN;

Peptide Innovations, Texas) is generally between about 0.4 mg/ml to about 1.5 mg/ml, preferably near 1.5 mg/ml. However, any bone morphogenetic protein is contemplated including bone morphogenetic proteins designated as BMP-1 through BMP-13, CDMP1 and CDMP2, and various growth factors know in the art to be beneficial for the induction of bone growth and tissue regeneration. BMPs are available from Genetics Institute, Inc., Cambridge, Massachusetts and may also be prepared by one skilled in the art as described in U.S. Patent Nos. 5,187,076 to Wozney et al.; 5,366,875 to Wozney et al.; 4,877,864 to Wang et al.; 5,108,922 to Wang et al.; 5,116,738 to Wang et al.; 5,013,649 to Wang et al.; 5,106,748 to Wozney et al.; and PCT Patent Nos. WO93/00432 to Wozney et al.; WO94/26893 to Celeste et al.; and WO94/26892 to Celeste et al. All osteoinductive factors are contemplated whether obtained as above or isolated from bone or other sources. Methods for isolating bone morphogenic protein from bone are described in U.S. Patent No. 4,294,753 to Urist and Urist et al., 81 PNAS 371, 1984.

The choice of carrier material for the osteogenic composition is based on the application desired, biocompatibility, biodegradability, and interface properties. The bone growth inducing composition can be introduced into the pores of the bone material in any suitable manner. For example, the composition may be injected into the pores of the graft. In other embodiments, the composition is dripped onto the graft or the graft is soaked in or sprayed with a solution containing an effective amount of the composition to stimulate osteoinduction. Alternatively, the osteogenic composition is infused into the bone under elevated or reduced pressure, or both. In any event, the pores of the RAB matrix are exposed to the composition for a period of time sufficient to allow the osteogenic composition to thoroughly soak, coat and infuse into the graft. The osteogenic factor, preferably a BMP, may be provided in freeze-dried form and reconstituted in a pharmaceutically acceptable liquid or gel carrier such as sterile water, physiological saline or any other suitable carrier. The carrier may be any suitable medium capable of delivering the proteins, cells or nucleic acids to the RAB graft. Preferably the medium is supplemented with a buffer solution as is known in the art. In one specific embodiment of the invention, rhBMP-2 is suspended or admixed in a carrier, such as MFR buffer, water, saline, liquid collagen or injectable bicalcium phosphate. In a preferred

embodiment, BMP is applied to the pores of the graft and then lypholized or freeze-dried. The graft-BMP composition can then be stored in a sterile container, at room temperature, or at decreased temperatures for storage and transport. Alternatively, the osteoinductive protein, cells or nucleic acids can be added at the time of surgery.

Other osteoinductive protein carriers are available to deliver proteins to a chamber defined within the bone material or to locations around the implantation site of the bone material. Potential carriers include calcium sulphates, polylactic acids, polyanhydrides, collagen, calcium phosphates, polyesters, polyphoazines, polyamines, polycarbonates, and the like, and demineralized bone. The carrier may be any suitable carrier capable of delivering the proteins. Most preferably, the carrier is capable of being eventually resorbed into the body. One preferred carrier is an absorbable collagen sponge marketed by Integra LifeSciences Corporation under the trade name Helistat® Absorbable Collagen Hemostatic Agent, (a biologically derived bovine achilles tendon collagen). Another preferred carrier is an open cell polylactic acid polymer (OPLA). Other potential matrices for the compositions may be biodegradable and chemically defined calcium sulfates, calcium phosphates such as tricalcium phosphate (TCP) and hydroxyapatite (HA) and including injectable bicalcium phosphates (BCP), and polyanhydrides. Other potential materials are biodegradable and are biologically derived, such as bone or dermal collagen. Further matrices are comprised of pure proteins or extracellular matrix components. The osteoinductive material may also be an admixture of BMP and a polymeric acrylic ester carrier, such as polymethylmethacrylate, polyvinylacetate, polyhydroxyethyl methacrylate, and the like. For packing the chambers of the spacers of the present invention, the carriers are preferably provided as a sponge which can be compressed into the chamber or as strips or sheets which may be folded to conform to the chamber. Preferably, the carrier has a width and length which are each slightly greater than the width and length of the chamber. In the most preferred embodiments, the carrier is soaked with a rhBMP-2 solution and then compressed into the chamber. The sponge is held within the chamber by the compressive forces provided by the sponge against the wall of the dowel. It may be preferable for the carrier to extend out of the openings of the chamber to facilitate contact of the osteogenic composition

with the highly vascularized tissue surrounding the fusion site. The carrier can also be provided in several strips sized to fit within the chamber. The strips can be placed one against another to fill the interior. As with the folded sheet, the strips can be arranged within the spacer in several orientations. Preferably, the osteogenic material, whether provided in a sponge, a single folded sheet or in several overlapping strips, has a length corresponding to the length and width of the chamber.

Another preferred carrier is a biphasic calcium phosphate ceramic. Hydroxyapatite/tricalcium phosphate ceramics are useful as carriers because of their desirable bioactive properties and degradation rates in vivo. A preferred ratio of hydroxyapatite to tricalcium phosphate is between about 0:100 and about 65:35. Any size or shape ceramic carrier which will fit into the chambers defined in the load-bearing member are contemplated. Ceramic blocks are commercially available from Sofamor Danek Group, B. P. 4-62180 Rang-du-Fliers, France and Bioland, 132 Route d:Espagne, 31100 Toulouse, France. Of course, rectangular and other suitable shapes are contemplated. The osteoinductive factor is introduced into the carrier in any suitable manner. For example, the carrier may be soaked in a solution containing the factor.

The present invention also provides spacers for maintaining a space between adjacent bones. The spacers include a body composed of RAB graft in combination with a bone growth factor. The bone source is any suitable bone material preferably of vertebrate origin, including tibial, fibial, humeral, iliac, etc. The RAB graft bodies of this invention include flat spacers, bone dowels, cortical rings, bone chips and any other suitably shaped bone piece. A preferred body is obtained from the diaphysis of a long bone having a medullary canal which forms a natural chamber in the graft.

In one specific embodiment depicted in Figure 1, the invention provides a spacer 10 for maintaining a space between adjacent bones in a patient. The spacer 10 includes a load-bearing member or body 11 sized and shaped to fit within the space. The body 11 is preferably composed of a natural RAB material which has been processed to remove associated non-collagenous bone proteins. The bone material contains native collagen

materials and naturally associated bone minerals but is substantially free from native non-collagenous protein. The chemical composition of the bone material allows it to resiliently retain a shaped body. The shape of the body is preferably formed, and the body machined to have desired surface features, before the bone material is processed according to the methods of this invention. However, in some embodiments a mass of bone is treated as disclosed herein, and then is shaped or machined to form a particular body.

Referring now to Figures 1 and 2, in some embodiments, the body 11 is shaped as a dowel. Dowel shaped bodies are sometimes preferred when the bones are vertebrae to be fused. The dowel 10 includes a wall 12 sized for engagement within the intervertebral space (IVS) to maintain the IVS in proper physiologic orientation. The wall 12 defines an outer engaging surface 13 for contacting the adjacent vertebrae. The wall 12 is preferably cylindrical, so that the bone dowel 10 has a diameter d which is larger than the height h of the IVS between adjacent vertebrae V or the height of the space between the lowest lumbar vertebrae L5 and the sacrum S as depicted in Figure 2.

In another embodiment depicted in Figure 3, the body is a bone dowel 20 which includes a wall 22 having an engagement surface 23. The wall 22 defines a chamber 25 therethrough. Preferably, the load-bearing member is a bone graft obtained from the diaphysis of a long bone having a medullary canal which forms the chamber 25. Such dowels are available from Regeneration Technologies, Inc., 1 Innovation Drive, Alachua, Florida 32615. The chamber 25 can be packed with an osteogenic composition to stimulate osteoinduction. The chamber 25 is preferably defined through a pair of outer engaging surfaces 23 so that the composition has maximum contact with the endplates of the adjacent vertebrae. Referring now to FIG. 4, the spacer 20 preferably includes a solid protective wall 26 which is positionable to protect the spinal cord from escape or leakage of material packed within the chamber 25. In anterior approaches, the protective wall 26 is posterior. Preferably, the osteogenic composition has a length which is greater than the length of the chamber (Figures 5 and 6) and the composition is disposed within the chamber 25 to contact the end plates of adjacent vertebrae when the spacer 20 is

implanted between the vertebrae. This provides better contact of the composition with the end plates to stimulate osteoinduction.

Various features can be machined on the outer surfaces of the dowels of this invention. In one embodiment shown in Figure 3, the dowel 20 includes an outer engaging surface 23 defining threads 24. Referring again to Figure 1, in some embodiments, the dowel 10 is provided with a tool-engaging hole 19 in a wall 18 opposite the solid protective wall 16. The tool engaging hole 19 is provided in a surface of the dowel which is adjacent the surgeon and opposite the initial thread 17. For an anterior procedure, the tool engaging tool hole 19 would be provided in the anterior surface of the dowel 10. Other machined features are contemplated in the outer or bone engaging surfaces 23. Such machine features include surface roughenings such as knurlings and ratchetings.

The spacers of this invention can be inserted using conventional techniques and known tools. In accordance with additional aspects of the present invention, methods for implanting an interbody fusion spacer, such as the spacer 20, are contemplated. The spacers of this invention can also be inserted using laporoscopic technology as described in Sofamor Danek USA's Laproscooic Bone Dowel Surgical Technique, 1995, 1800 Pyramid Place, Memphis, Tennessee 38132,1-800-933-2635. Devices of this invention can be conveniently incorporated into Sofamor Danek's laproscopic bone dowel system that facilitates anterior interbody fusions with an approach that is much less surgically morbid than the standard open anterior retroperitoneal approaches. This system includes templates, trephines, dilators, reamers, ports and other devices required for laproscopic dowel insertion.

The body may also include other shapes such as cortical rings as shown in Figure 7. Such cortical rings 50 are obtained by a cross-sectional slice of the diaphysis of a long bone and include a superior surface 51 and an inferior surface 52. The graft shown in Figure 7 includes an outer surface 53 which is adjacent and between the superior 51 and inferior 52 surfaces. In one embodiment bone growth through-holes 53a are defined through the outer surface 53 to facilitate fusion. The holes 53a allow mesenchymal stem

cells to creep in and bone growth protein to diffuse out of the graft. This facilitates bone graft incorporation and possibly accelerates fusion by forming anterior and lateral bone bridging outside and through the device. In another embodiment the outer surface 53 defines a tool-engaging hole 54 for receiving an implanting tool. In a preferred embodiment, at least one of the superior and/or inferior surfaces 51, 52 are roughened for gripping the end plates of the adjacent vertebrae. The surface roughenings may include teeth 56 on ring 50' as shown in Figure 8 or waffle pattern 57 as shown on ring 50" in Figure 9. When cortical rings are used as the graft material the ring 50 may be trimmed for a more uniform geometry as shown in Figure 7 or left in place as shown in Figure 9.

The graft can also be formed into a square shape to be conveniently incorporated into current surgical procedures such as, the Smith-Robinson technique for cervical fusion (Smith, M.D., G.W. and R.A. Robinson, M.D., "The Treatment of Certain Cervical-Spine Disorders By Anterior Removal Of The Intervertebral Disc And Interbody Fusion", J. Bone And Joint Surgery, 40-A:607-624 (1958) and Cloward, M.D., R.B., "The Anterior Approach For Removal Of Ruptured Cervical Disks", in meeting of the Harvey Cushing Society, Washington, D.C., April 22, 1958). In such procedures, the surgeon prepares the endplates of the adjacent vertebral bodies to accept a graft after the disc has been removed. The endplates are generally prepared to be parallel surfaces with a high-speed burr. The surgeon then typically sculpts the graft to fit tightly between the bone surfaces so that the graft is held by compression between the vertebral bodies. The bone graft is intended to provide structural support and promote bone ingrowth to achieve a solid fusion of the affected joint. The spacers of this invention avoid the need for this graft sculpting as spacers of known size and dimensions are provided. This invention also avoids the need for a donor surgery because the osteoinductive properties of autograft are provided by the allograft or xenograft RAB implants prepared according to the present invention. The spacers can be combined with osteoinductive materials that make allograft or xenograft RAB implants osteoinductive. Therefore, the spacers of this invention speed the patient's recovery by reducing surgical time, avoiding a painful donor surgery and inducing quicker fusion. The following specific examples are provided for

purposes of illustrating the invention, and no limitations on the invention are intended thereby.

EXAMPLE 1

REMOVAL OF ANTIGENS FROM BONE GRAFT MATERIAL

This procedure, and variations on the specifics thereof, is employed to remove non-collagenous protein from bone graft materials. The bone graft material may be allograft or xenograft, selected from bovine, porcine, equine, ovine, canine or the like. This procedure removes proteins, fats, polysaccharides, glycosaminoglycans and other non-collagenous antigens from bone matrix, and may be conducted in any order of steps, although carrying the process out in the order provided herein has provided consistently excellent results:

1. Peroxide Treatment:

This procedure is followed to de-fat the bone tissue, to remove blood and other proteins, and to inactivate microorganisms that might be present in or on the bone. Prior to initiating this treatment, the bone was cleaned of any attached adventitious tissue.

a. The bone tissue was placed into a container, covered with peroxide solution, and permitted to soak with agitation, sonication or both for about 15 minutes.

b. The peroxide solution and removed debris was decanted, and the bone tissue was rinsed with warm sterile water.

Treatment at this stage with a TNBP/Triton X-100 or like solutions, such as hydrogen peroxide/SDS helps to remove additional non-structural proteins and residual fat.

2. Acetone Treatment:

This procedure was followed to remove residual fatty tissue:

a. The bone tissue was placed into a container, covered with acetone, and heated to between about 35 to 40 degrees centigrade, and permitted to soak with agitation for about 15 minutes. This step was repeated until no fat was visible in the solution after being allowed to cool (three to five cycles is usually adequate).

b. The bone tissue was then rinsed with sterile water and permitted to dry.

Variations on this treatment may include use of 99% isopropanol, hexane, and combinations of these solvents. Treatment of the graft at this or a different stage of the process with acetic or other acid (acetic acid, hydrochloric acid, hydrofluoric acid, phosphoric acid, citric acid, formic acid, butyric acid, or mixtures thereof), is useful to produce a slightly demineralized bone graft of reduced antigenicity, with concomitant effects on the graft strength, growth factor binding capacity, resorbability, removal of acid soluble proteins and loosely associated collagens, and further reductions in antigenicity. We have discovered that reduction in the mineral content of between about 0 to about 25%, or between about 1 to about 10% or even as little as 1% to 5% as compared to the normal bone mineral content confers significant advantages on the reduced antigenicity bone composition. The guiding principle in the level of demineralization that should be conducted is to remove as much mineral as possible, without at the same time reducing the compressive strength of the bone. In order to achieve uniform, limited demineralization, the RAB is preferably contacted for about thirty minutes with acid, e.g. 1% acetic acid, with the acid being introduced into an evacuated chamber containing the RAB, such that uniform acid penetration occurs. If inorganic acids are used, e.g. HCl, the acid strength or period of acid contact should be reduced, to avoid complete demineralization of the RAB. We have found that limited removal of even as little as 1-2% of the normal bone mineral content results in greater predictability (reduced scatter in shear stress measurments) in the strength of bone grafts thus treated. Additional benefits of this treatment include dissolution of acid soluble

proteins, efficient removal of SDS or other ionic solvents or contaminants, enhanced binding of growth factors, reduced time to remodel implanted bone, and further reduction in antigenicity.

3. Urea Treatment:

This procedure was followed in order to remove associated non-collagenous proteins from the bone tissue.

a. The tissue was transferred to a container sufficient to contain the tissue and a large excess volume (approximately five-fold) of urea solution (6 M).

b. Non-collagenous proteins were extracted from the bone tissue for approximately 48 hours, with agitation.

c. The urea/protein solution was then decanted, and the bone tissue was rinsed with sterile water, several times(about three) using at least a two-fold volume of water. Each rinse was permitted to continue with agitation for about 20 minutes.

d. A final water wash was conducted for 24 hours with agitation, followed by decantation of the water and freeze-drying of the tissue.

The foregoing procedure was conducted with bovine bone blocks and cancellous chips. Bone cubes of 1 cm were cut from bovine condyles. As a final sterilization step, the thus treated bone was subjected to lyophilization and then gamma irradiation. Bone implant material treated according to this procedure was implanted into a primate model. Little or no adverse immune response (swelling, inflammation) was detected. Furthermore, bone implant treated in this manner was soaked with bone morphogenic protein and implanted in a primate model. Excellent induction of new bone growth into and around the implant bovine bone was detected, without adverse immune response. Based on the success achieved in using bone implant prepared as described herein for delivery of growth factors in the form of active protein, success in delivering cells expressing growth factors, or nucleic acids actively encoding growth factors is expected. Allograft or xenograft bone infused or coated with cells, recombinant or natural, which produce growth factors,

or nucleic acid constructs, such as those disclosed in US Patent No. 5,763,416, hereby incorporated by reference, or WO 99/06563, also hereby incorporated, are anticipated to actively induced bone growth without induction of adverse immune responses.

Alternatives to the above-described treatment includes the use of guanidinium hydrochloride, TritonX-100, Tween, TNBP and the like, optionally including combinations of chaotropic agents and surfactants such as SDS (sodium dodecyl sulfate). Examples of conditions for use of these agents include use of 4 M guanidinium hydrochloride, and 1% TNBP/TritonX-100.

EXAMPLE 2

PREPARATION OF DIAPHYSIAL CORTICAL BONE DOWEL

A consenting donor (i.e., donor card or other form of acceptance to serve as a donor) was screened for a wide variety of communicable diseases and pathogens, including human immunodeficiency virus, cytomegalovirus, hepatitis B, hepatitis c and several other pathogens. These tests may be conducted by any of a number of means conventional in the art, including but not limited to ELISA assays, PCR assays, or hemagglutination. Such testing follows the requirements of: (i) American Association of Tissue Banks, Technical Manual for Tissue Banking, Technical Manual - Musculoskeletal Tissues, pages M19-M20; (ii) The Food and Drug Administration, Interim Rule, Federal Register/Vol. 50, No. 238/Tuesday, December 14, 1993/Rules and Regulations/65517, D. Infectious Disease Testing and Donor Screening; (iii) MMWR/Vol. 43/No. RR-8, Guidelines for Preventing Transmission of Human Immunodeficiency Virus Through Transplantation of Human Tissue and Organs, pages 4-7; (iv) Florida Administrative Weekly, Vol. 10, No. 34, August 21, 1992, 59A-1.OOI-O14 59A-1.005(12)(c), F.A.C., (12)(a)-(h), 59A-1.005(15), F.A.C., (4)(a)-(8). In addition to a battery of standard biochemical assays, the donor, or their next of kin, was interviewed to ascertain whether the donor engaged in any of a number of high risk behaviors such as having multiple sexual partners, suffering from hemophilia, engaging in intravenous drug use etc. After

the donor was ascertained to be acceptable, the bones useful for obtention of the dowels were recovered and cleaned.

A dowel was obtained as a transverse plug from the diaphysis of a long bone using a diamond tipped cutting bit which was water cleaned and cooled. The bit was commercially available (Starlite, Inc) and had a generally circular nature and an internal vacant diameter between about 10 mm to about 20 mm. The machine for obtention of endo- and cortical dowels consisted of a pneumatic driven miniature lathe which is fabricated from stainless steel and anodized aluminum. It has a spring-loaded carriage which travels parallel to the cutter. The carriage rides on two runners which are 1.0 inch stainless rods and has a travel distance of approximately 8.0 inches. One runner has set pin holes on the running rod which will stop the carriage from moving when the set pin is placed into the desired hole. The carriage is moveable from side to side with a knob which has graduations in metric and in English. This allows the graft to be positioned. On this carriage is a vice which clamps the graft and holds it in place while the dowel is being cut. The vice has a cut out area in the jaws to allow clearance for the cutter. The lathe has a drive system which is a pneumatic motor with a valve controller which allows a desired RPM to be set.

First, the carriage is manually pulled back and locked in place with a set pin. Second, the graft is loaded into the vice and is aligned with the cutter. Third, the machine is started and the RPM is set, by using a knob on the valve control. Fourth, the set pin, which allows the graft to be loaded onto the cutter to cut the dowel. Once the cutter has cut all the way through the graft the carriage will stop on a set pin. Fifth, sterile water is used to eject dowel out of the cutter. It is fully autoclavable and has a stainless steel vice and/or clamping fixture to hold grafts for cutting dowels. The graft can be positioned to within 0.001" of an inch which creates dowel uniformity during the cutting process.

The cutter used in conjunction with the above machine can produce dowels ranging from 5 mm to 30 mm diameters and the sizes of the cutters are 10.6 mm; 11.0 mm; 12.0 mm; 13.0 mm; 14.0 mm; 16.0 mm; and 18.0 mm. The composition of the cutters is stainless

steel with a diamond powder-cutting surface which produces a very smooth surface on the wall of the dowels. In addition, sterile water is used to cool and remove debris from graft and/or dowel as the dowel is being cut (hydro infusion). The water travels down through the center of the cutter to irrigate as well as clean the dowel under pressure. In addition, the water aids in ejecting the dowel from the cutter.

The marrow was then removed from the medullary canal of the dowel and the cavity cleaned to create a chamber. The chamber interior may be scraped or machined as desired and may be filled with desired osteogenic materials, including allograft, autograft, ceramic, growth factors and the like. The final machined product may be stored, frozen or freeze-dried and vacuum-sealed for later use.

EXAMPLE 3

THREADING OF DOWELS

A diaphysial cortical bone dowel is prepared as described above. The plug is then machined, preferably in a class 10 clean room, to the dimensions desired. The machining is preferably conducted on a lathe such as a jeweler's lathe or machining tools may be specifically designed and adapted for this purpose. A hole is then drilled through the anterior wall of the dowel. The hole is then tapped to receive a threaded insertion tool.

EXAMPLE 4

PREPARATION OF RAB DOWEL-rhBMP-2 COMPOSITE BY DRIPPING

A threaded RAB dowel is obtained through the methods described above. A vial containing 4.0 mg of lypholized rhBMP-2 (Genetics Institute) is constituted with 1 mL sterile water (Abbott Laboratories) for injection to obtain a 4.0 mg/mL solution as follows:

1. Using a 3-cc syringe and 22G needle, slowly inject 1.0 mL sterile water for injection into the vial containing lypholized rhBMP-2.

2. Gently swirl the vial until a clear solution is obtained. Do not shake.

The dilution scheme below is followed to obtain the appropriate rhBMP-2 concentration. This dilution provides sufficient volume for two dowels. The dilutions are performed as follows:

1. Using a 5-cc syringe, transfer 4.0 mL of MFR 906 buffer (Genetics Institute) into a sterile vial.
2. Using a 1-cc syringe, transfer 0.70 mL reconstituted rhBMP-2 into the vial containing the buffer.
3. Gently swirl to mix.

DILUTION SCHEME

INITIAL rhBMP-2 CONCENTRATION (mg/mL)	rhBMP-2 VOLUME (mL)	MFR-842 VOLUME (mL)	FINAL rhBMP-2 CONCENTRATION (mg/mL)
4.0	0.7	4.0	0.6

1. Using a 3-cc syringe and 22G needle, slowly drip 2.0 mL of 0.60 mg/mL rhBMP-2 solution onto the Bone Dowel.
2. Implant immediately.

As an alternative to the above, a RAB implant is lyophilized, and then brought into contact with a solution containing osteogenic proteins, growth factors, nucleic acids and the like, in order to reconstitute the lyophilized RAB. In the process of being reconstituted, the RAB draws the growth factors or other osteogenic compositions, natural or recombinant, into the interstices of the RAB matrix.

EXAMPLE 5

PREPARAION OF RAB ALLOGRAFT OR XENOGRAFT BONE-BMP COMPOSITE BY SOAKING

1. Freeze dried rhBMP-2 is reconstituted with sterile water for injection as in Example 4.
2. A sterile RAB allograft or xenograft bone dowel is transferred to a sterile "soaking" container.
3. Reconstituted rhBMP-2 is added to the soaking container so that the allograft is completely submersed in a BMP solution.
4. The RAB allograft or xenograft bone dowel is allowed to soak in the rhBMP-2 solution for 30-60 minutes so that the graft absorbs the protein.

To enhance the efficiency of loading of BMPs or other growth factors, the RAB allograft or xenograft is contacted with such factors under vacuum with swirling for about 15 minutes.

EXAMPLE 6

BONE DOWEL PACKED WITH BMP-2/COLLAGEN COMPOSITION

A threaded RAB dowel is obtained through the methods of Examples 1-5. A vial containing 4 .0 mg of lypholized rhBMP-2 (Genetics Institute) is constituted with 1 mL sterile water (Abbott Laboratories) for injection to obtain a 4.0 mg/mL solution as follows:

1. Using a 3-cc syringe and 22G needle, slowly inject 1.0 mL sterile water for injection into the vial containing lypholized rhBMP-2.
2. Gently swirl the vial until a clear solution is obtained. Do not shake. The dilution scheme below is followed to obtain the appropriate rhBMP-2 concentration. The dilutions are performed as follows:
1. Using a 3-cc syringe, transfer 2.5 mL of MFR-842 buffer (Genetics Institute) into a sterile vial.

2. Using a 1-cc syringe, transfer 0.30 mL of 4.0 mg/mL reconstituted rhBMP-2 into the vial containing the buffer.
3. Gently swirl to mix.

DILUTION SCHEME

INITIAL rhBMP-2 CONCENTRATION (mg/mL)	rhBMP-2 VOLUME (mL)	MFR-842 VOLUME (mL)	FINAL rhBMP-2 CONCENTRATION (mg/mL)
4.0	0.3	2.5	0.43

The rhBMP-2 solution is applied to a Helistat sponge (Genetics Institute) as follows:
1. Using sterile forceps and scissors, cut a 7.5 cm x 2.0 cm strip of Helistat off of a 7.5 x 10 cm (3" x 4") sponge.
2. Using a 1-cc syringe with a 22-G needle, slowly drip approximately 0.8 mL of 0.43 mg/mL rhBMP-2 solution uniformly onto the Helistat sheet.
3. Using sterile forceps, loosely pack the sponge into the chamber of the RAB dowel.
4. Using a 1-cc syringe with a 22-G needle, inject the remaining 0.8 mL of 0.43 mg/mL rhBMP-2 into the sponge in the RAB dowel through the openings of the chamber.
5. Implant immediately.

EXAMPLE 7

RAB DOWEL PACKED rhBMP-2/HA/TCP COMPOSITION

A threaded RAB dowel is obtained through the methods of Examples 1-5. A vial containing 4.0 mg of lypholized rhBMP-2 (Genetics Institute) is constituted with 1 mL sterile water (Abbott Laboratories) for injection to obtain a 4.0 mg/mL solution as follows:

1. Using a 3-cc syringe and 22G needle, slowly inject 1.0 mL sterile water for injection into the vial containing lypholized rhBMP-2.

2. Gently swirl the vial until a clear solution is obtained. Do not shake. A cylindrical block of biphasic hydroxyapatite/tricalcium phosphate (Bioland) is wetted with a 0.4 mg/mL rhBMP-2 solution. The BMP-ceramic block is packed into the chamber of the RAB dowel and the thus packed RAB dowel is then implanted.

EXAMPLE 8

RAB ALLOGRAFT OR XENOGRAFT BONE CHIP-COMPOSITE PREPARATION

1. Allograft or xenograft bone chips are harvested, processed and prepared according to Example 1 to produce RAB allograft or xenograft bone chips.

2. Freeze dried rhBMP-2 is reconstituted with sterile water for injection as described in Example 4.

3. The sterile RAB allograft or xenograft bone chips are transferred to the sterile "soaking" container. Preferably, the RAB bone chips are first lyophilized, so that upon contact with the BMP solution, the bone chips reconstitute, thereby soaking up the BMP solution into the interstices of the chips.

4. Reconstituted rhBMP-2 is placed into the soaking container so that the RAB allograft or xenograft is completely submersed.

5. The RAB allograft or xenograft bone chips are soaked in the rhBMP-2 solution for 30-60 minutes.

6. Using sterile forceps, the RAB allograft or xenograft bone chips are removed from the soaking container and placed into the posterolateral gutters of the level of the spine to be fused, or into any other bony location where bone fusion or repair is desired.

This procedure may be employed to make a gelatin sponge by injecting gelatin into bone chips prepared as described above and then lyophilizing the composition. As with Example 5, the efficiency of BMP or growth factor loading is enhanced when contact is made with the RAB under vacuum.

EXAMPLE 9
PREPARATION OF RAB CORTICAL RING-COMPOSITES

A cortical ring is obtained as a cross-sectional slice of the diaphysis of a human long bone and then prepared using the methods described in Example 1 to produce a RAB cortical ring. The RAB ring is fashioned into a square hollow ring. The ring is packed with an osteogenic composition as described in the foregoing EXAMPLES.

EXAMPLE 10
RAB SPACERS

A RAB D-shaped cervical spacer is obtained as a cross-sectional slice of a diaphysis of a long bone and treated according to the method of Example 1. The exterior surfaces of the walls are formed by machining the slice to a D-shape. The engaging surfaces of the spacer are provided with knurlings by a standard milling machine. A hole is then drilled through the anterior wall of the spacer. The hole is then tapped to engage a threaded insertion tool. The chamber of the spacer is then packed with an osteogenic composition as described in the foregoing EXAMPLES.

EXAMPLE 11
ANTEROR INTERBODY CERVICAL FUSION

The cervical spine is approached anteriorly according to known surgical techniques. The RAB composite material is placed within the interdiscal space.

EXAMPLE 12

POSTEROLATERAL FUSION

The spine is approached posterolaterially according to known surgical techniques. The RAB composite material of this invention is placed between portions of adjacent vertebrae.

EXAMPLE 13

USE OF RAB COMPOSITE WITH BINDING MATRIX

Processed RAB allograft or xenograft is added to a binding matrix to hold the allograft chips together improving their handling characteristics. RAB chips prepared according to Example 5 are lyophilized and then mixed with gelatin and water to form a paste or slurry, then, optionally, freeze dried into a sheet or any other desired form. At the time of surgery the surgeon hydrates the gelatin RAB allograft/xenograft composite with an osteoinductive protein solution. Alternative binding matrix materials include glycosaminoglycans, hyaluronic acid, polymers, proteins and other suitable materials. With or without added demineralized bone matrix, the compositions described herein may have applications in diverse areas of the orthopedic arts. For example, pre-formed shapes may be prepared using appropriately proportioned quantities of RAB, RAB that has been demineralized (DRAB), gelatin, growth factors and the like. Similar compositions may be envisioned for RACs and RATs. Compositions wherein gelatin is present at a sufficiently high concentration that the composition is in a semi-liquid, malleable solid or viscous liquid above normal body temperature of a recipient, but becomes a gel or solid at normal body temperature, upon implantation into the recipient are highly desirable. For such applications, gelatin concentrations of between about one to twenty-five percent are typically sufficient, depending on the average molecular weight of the gelatin employed in such compositions. In addition, compositions wherein gelatin is present at a sufficiently high concentration that the composition is a solid at a temperature above normal body temperature of a recipient but is a malleable solid at a slightly higher temperature, such that a solid of substantially any desired form may be

made at the higher temperature, and upon implantation into a recipient, the composition maintains the formed shape, are also highly desirable. Typically, gelatin concentrations of between about ten and forty percent are sufficient for this purpose, depending on the molecular weight of the gelatin employed for such compositions.

EXAMPLE 14
CHARACTERISTICS AND APPLICATIONS FOR RAB

The combination of a bone growth factor with a RAB graft provides superior results as compared with other known implant materials. Quicker fusion rates provide enhanced mechanical strength sooner. The RAB of this invention is an excellent protein carrier which provides controlled release of BMP or other osteogenic compositions, including growth factors, cartilage derived morphogenic proteins, nucleic acids encoding BMPs or other growth factors, to the fusion site. The presence of structural collagen and the natural mineral structure of bone results in an elasticity and radioopacity which is identical or nearly identical to bone. The material has sufficient resilience and elasticity to retain a formed body and yet remains rigid enough to maintain an open space between bone portions to result in a fusion mass.

EXAMPLE 15
REDUCED ANTIGENICITY CARTILAGE AND OTHER TISSUES

In a manner similar to that used for preparation of RAB implants, reduced antigenicity cartilage (RAC) and reduced antigenicity tissues (RAT) may be produced by treating such tissues with chaotropic agents, as described above for bone. The thus-treated cartilage and other tissues may then optionally be contacted with various growth factors, nucleic acids and cells, as described above for the RAB implants of this invention.

As discussed above, the goal of RAB/RAC/RAT production is to remove all unbound substances. This goal is achieved according to the methods disclosed herein for such varied tissues as bone, cartilage, skin, fascia, dura and the like.

While the invention has been illustrated and described in detail in the drawings and foregoing description, the same is to be considered as illustrative and not restrictive in character, it being understood that only the preferred embodiments and best mode have been shown and described and that all changes and modifications that come within the spirit of the invention are desired to be protected.

What is claimed is:

1. A reduced antigenicity tissue (RAT) graft composition, comprising natural tissue material which has been processed to remove substantially all associated non-collagenous or non-structural collagen proteins, said material containing native collagen materials and (a) being demineralized or (b) retaining naturally associated minerals.

2. The reduced antigenicity tissue (RAT) of claim 1 wherein said tissue is reduced antigenicity bone (RAB) or reduced antigenicity cartilage (RAC).

3. The RAB and RAC of claim 2 wherein substantially all non-collagenous bone or cartilage proteins and substantially all non-structural collagenous proteins have been removed.

4. The RAB according to claim 3 prepared by a process comprising removing associated non-bone adventitious materials from a bone graft to provide a cleaned bone graft, contacting the cleaned bone graft with defatting solutions to provide a cleaned defatted bone graft, and contacting said cleaned defatted bone graft with a chaotropic agent to remove non-collagenous or non-structural collagen proteins to provide said RAB.

5. The RAB according to claim 4 wherein said chaotropic agent is selected from urea, guanidinium hydrochloride, Tween, TritonX-100, TNBP, SDS, and mixtures of these agents.

6. The RAB according to claim 2 further comprising an effective amount to stimulate bone growth of an osteogenic composition incorporated within said RAB material.

7. The RAB graft of claim 6 wherein said RAB is processed at temperatures no higher than about 250°C, and wherein said osteogenic composition comprises a recombinant or natural BMP, CDMP, TGF-beta, TGF-beta superfamily members, FGF, PDGF, P15, peptide, or a nucleic acid encoding BMP, peptide or CDMP.

8. The RAB of claim 2 wherein said bone is human, bovine, ovine, equine, porcine, or canine bone, or combinations thereof.

9. The RAT according to claim 1 machined to form spacers, pins, suture anchors, interference screws, demineralized bone implants, including but not limited to ligaments, oral maxilofacial plates, dowels, posterior lumbar interbody fusion implants, trauma screws and plates, fascia, dura, skin, pericardium (for dura, plura, shoulder patch and perioligaments), wedges, chips and pastes comprising reduced antigenicity bone, cartilage or other tissues, alone or in combination with growth factors, or nucleic acids encoding growth factors, including but not limited to bone morphogenetic proteins, cartilage derived morphogenetic proteins, tissue growth factor (beta1 and the like).

10. The spacer according to claim 9 for maintaining a space between a pair of adjacent vertebrae in a spine, comprising: a body sized and shaped to fit within the space, said body composed of reduced antigenicity bone, RAB, which has been processed to remove associated non-collagenous and non-structural collagen bone proteins, said bone material containing native collagen materials and naturally associated bone minerals.

11. The spacer according to claim 10 prepared by a process comprising removing associated non-bone adventitious materials from a bone graft to provide a cleaned bone graft, contacting the cleaned bone graft with defatting solutions to provide a cleaned defatted bone graft, and contacting said cleaned defatted bone graft with a chaotropic agent to remove non-collagenous or non-structural collagen proteins to provide said RAB, wherein said bone graft is either shaped to form said spacer

prior to said cleaning, defatting and contacting, or is shaped after said cleaning, defatting and contacting.

12. The spacer according to claim 11 further comprising an effective amount to stimulate bone growth of an osteogenic factor in combination with said RAB material.

13. The spacer of claim 12 wherein said body defines a superior wall for contacting a superior vertebra, an inferior wall for contacting an inferior vertebra and a lateral wall adjacent and between said superior wall and said inferior wall, said lateral wall defining a through hole.

14. The spacer of claim 13 wherein said body is derived from a femoral ring.

15. The spacer of claim 13 wherein said body is derived from a bone dowel.

16. The spacer of claim 13 wherein said walls define a chamber and said chamber is packed with a pharmaceutically acceptable carrier having said bone growth factor dispersed therein.

17. The spacer of claim 13 wherein said RAB material has dispersed therein said bone growth factor in a pharmaceutically acceptable carrier.

18. The spacer of claim 13 wherein said body is fully or partially resorbed after implantation no later than about five months.

19. The spacer of claim 13 wherein said body has approximately the radioopacity, after implantation, of the bones of the vertebrae between which said spacer is inserted.

20. The spacer of claim 13 wherein said bone graft is human, bovine, ovine, equine or canine bone.

21. A composition, comprising: processed bone material composed of bone minerals having a natural crystalline structure of bone and native collagen materials, said processed bone material being substantially free of non-collagenous bone proteins, and an effective amount to stimulate bone growth of an osteogenic factor within said material.

22. An elastic body consisting essentially of structural bone collagen and natural bone minerals in a natural configuration, substantially free of non-collagenous proteins and non-structural collagen protein in combination with an effective amount to stimulate bone growth of an osteogenic factor.

23. A surgical procedure for stabilizing a spine, comprising the steps of: exposing a portion of each of adjacent vertebrae requiring stabilization; and placing a processed bone material within an area between the portions of the adjacent vertebrae, the material composed of bone minerals having a natural crystalline structure of bone and native collagen materials, the processed bone material being substantially free of non-collagenous bone proteins, and an effective amount to stimulate bone growth of an osteogenic composition in combination with the material.

24. The surgical procedure of claim 23 wherein the bone material is formed into an elastic body defining a chamber into which is packed an osteogenic composition in a carrier.

25. The surgical procedure of claim 24 wherein the bone material has dispersed therein said osteogenic factor in a pharmaceutically acceptable carrier.

26. The procedure of claim 23 wherein the portions of the spine are at the posterolateral aspect of the spine.

27. The procedure of claim 26 wherein the material includes bone chips.

28. The composition of claim 21 wherein said composition is in the form of bone chips.

29. The composition of claim 28 further comprising a binding matrix, said chips disposed within said matrix.

30. The composition of claim 29 wherein said matrix includes gelatin.

31. The composition of claim 30 wherein said gelatin is present at a sufficiently high concentration that said composition is in a semi-liquid, malleable solid or viscous liquid above normal body temperature of a recipient, but becomes a gel or solid at normal body temperature, upon implantation into said recipient.

32. The composition of claim 30 wherein said gelatin is present at a sufficiently high concentration that said composition is a solid at a temperature above normal body temperature of a recipient but is a malleable solid at a slightly higher temperature, such that a solid of substantially any desired form may be made a the higher temperature, and upon implantation into said recipient, the composition maintains said shape.

33. A bone graft composition, comprising: natural bone material which has been processed to remove associated non-collagenous bone proteins, said bone material containing native collagen materials and naturally associated bone minerals and substantially free from native non-collagenous protein.

34. The composition of claim 33, wherein: said bone material is in the form of a body sized and shaped to form spacers, pins, suture anchors, interference screws, demineralized bone implants, including but not limited to ligaments, oral maxilofacial plates, dowels, posterior lumbar interbody fusion implants, trauma screws and plates, pericardium (for dura, plura, shoulder patch and perioligaments), wedges, chips and pastes comprising reduced antigenicity bone, cartilage or other tissues, alone or in combination with growth factors, or nucleic acids encoding growth factors, including but not limited to bone morphogenetic proteins, cartilage derived morphogenetic proteins, tissue growth factor (beta1 and the like).

35. The spacer according to claim 34 sized and shaped to fit within a space between a pair of adjacent vertebrae in a spine and wherein said body has approximately the radioopacity after implantation of the bones of the vertebrae.

36. The composition of claim 35, wherein said body defines a superior wall for contacting a first vertebra, an inferior wall for contacting a second vertebra, and a lateral wall adjacent and between said superior wall and said inferior wall, said lateral wall defining a through wall.

37. The composition of claim 36 wherein said body is derived from a femoral ring.

38. The composition of claim 36 wherein said body is derived from a bone dowel.

39. The composition of claim 36, wherein said walls define a chamber wherein said chamber is packed with a pharmaceutically acceptable carrier having an osteogenic factor dispersed therein.

40. The composition of claim 36 wherein said body is fully or partially resorbed after implantation no later than about five months.

41. The composition of claim 40 wherein said bone graft is human, bovine, ovine, equine, porcine or canine bone.

42. The RAT of claim 1 wherein said osteogenic composition is incorporated into said RAT by freeze-drying said RAT and reconstituting said RAT in a solution comprising said osteogenic composition.

43. The RAT of claim 42 wherein said RAT is RAB and wherein said osteogenic composition comprises nucleic acid.

44. The RAT of claim 1 wherein said osteogenic composition is incorporated into said RAT under vacuum or reduced pressure.

45. The RAT of claim 44 wherein said RAT is RAB and said osteogenic composition comprises nucleic acid.

46. The RAT according to claim 1 wherein said RAT is RAB which is contacted with acid.

47. The RAT according to claim 46 wherein said RAB is contacted with acetic acid, hydrochloric acid, hydrofluoric acid, phosphoric acid, citric acid, formic acid, butyric acid, or mixtures thereof, such that said RAB is demineralized to an extent between about 0 to 25% of the normal bone mineral content.

48. The RAB according to claim 47 wherein said RAB is demineralized to an extent between about 1 to 10% of the normal bone mineral content.

49. The RAB according to claim 48 wherein said RAB is demineralized to an extent between about 1 to about 5% of the normal bone mineral content.

50. A method for making a reduced antigenicity tissue for implantation into a recipient in need thereof which comprises:

(a) cleaning a tissue section of unwanted materials;

(b) contacting the thus cleaned tissue section with a chaotropic agent to remove non-structural proteins to produce a non-structural protein depleted tissue section; and

(c) contacting said non-structural protein depleted section with chemical or energetic agents sufficient to eliminate or inactivate microorganisms.

51. The method according to claim 50 wherein said tissue is bone, such that the thus produced tissue is reduced antigenicity bone.

52. The method according to claim 51 wherein said reduced antigenicity bone is further contacted, at any stage of the process for preparing said reduced antigenicity bone, with sufficient acid for a sufficient amount of time to produce a reduced antigenicity bone containing between with a mineral content that has been reduced by between about 0 to about 25% of the normal bone mineral content.

53. The method according to claim 52 wherein said reduced antigenicity bone is contacted with a biologically active agent selected from the group consisting of growth factors, nucleic acids, antibiotics, antineoplastics, antifungals, antivirals and combinations thereof under conditions sufficient to permit uptake of said biologically active agent into the matrix of said reduced antigenicity bone.

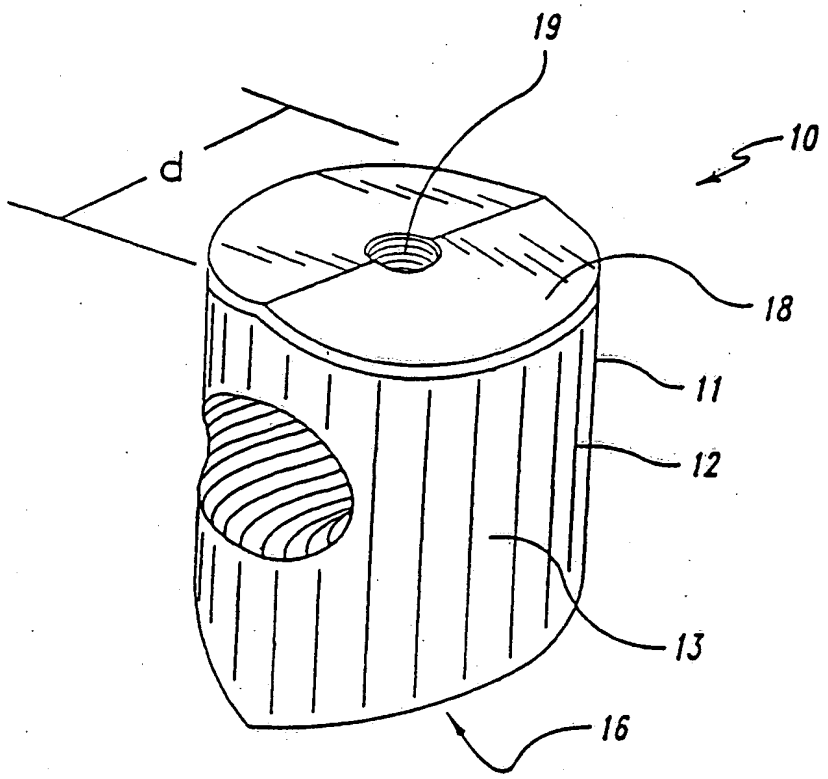

FIGURE 1

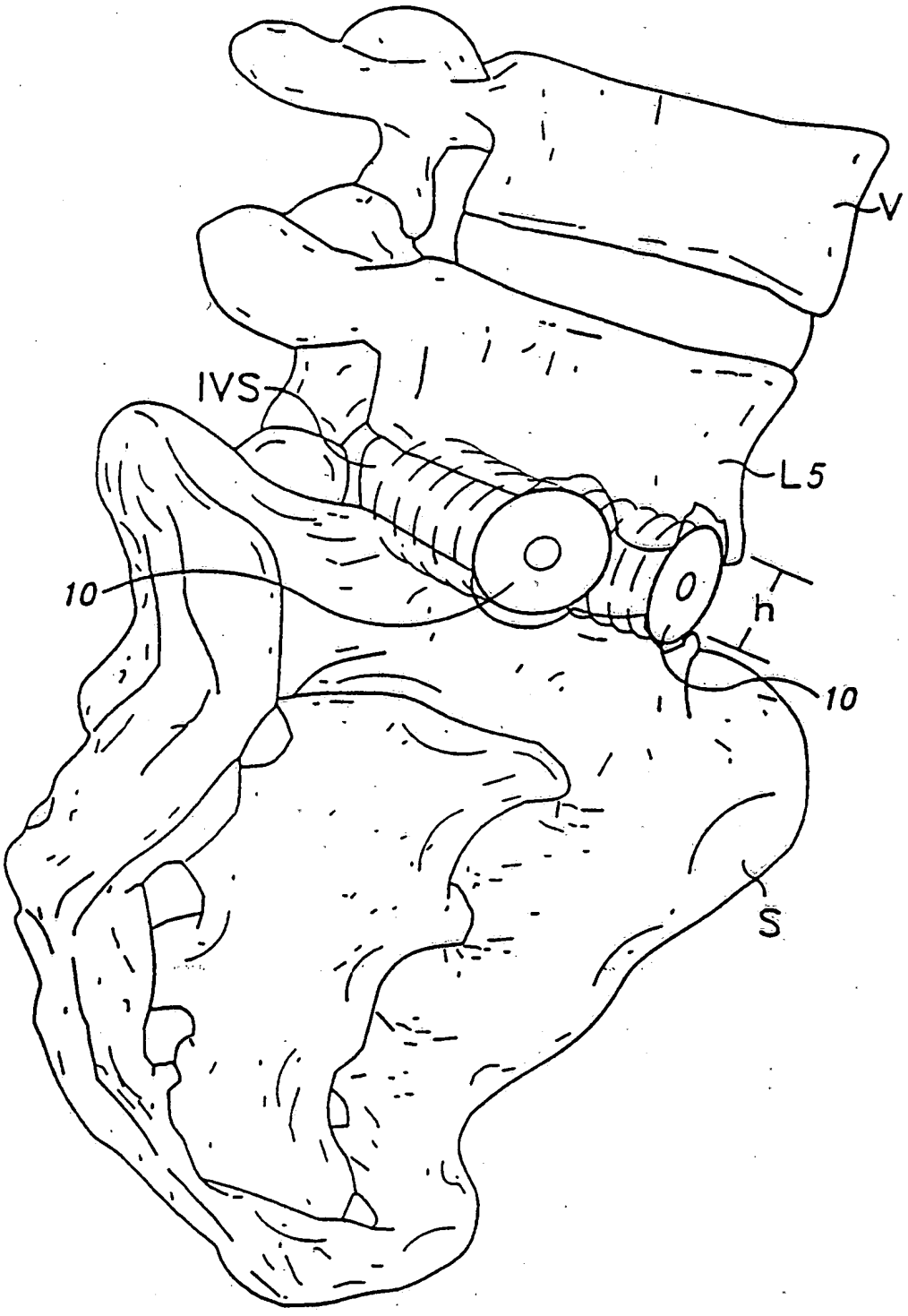

FIGURE 2

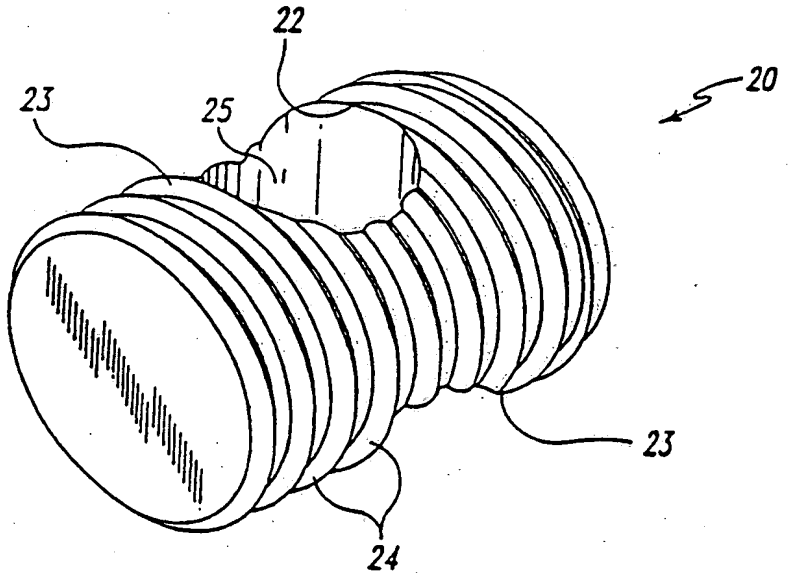

FIGURE 3

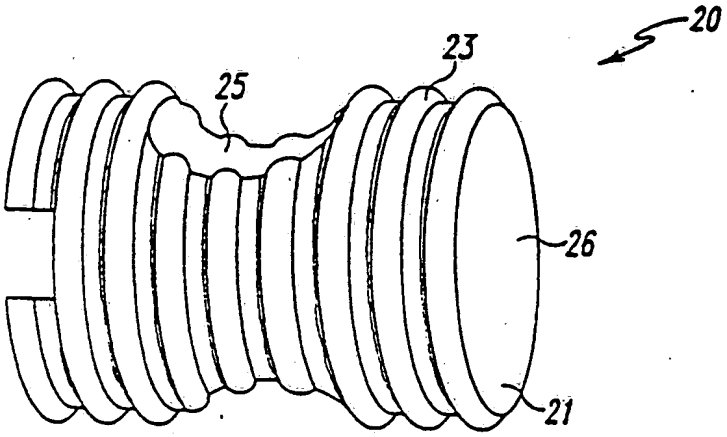

FIGURE 4

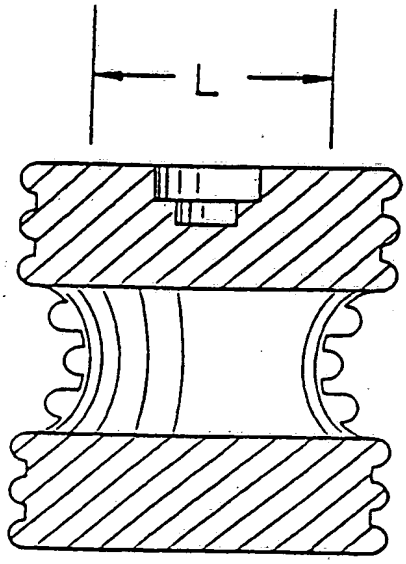

FIGURE 5

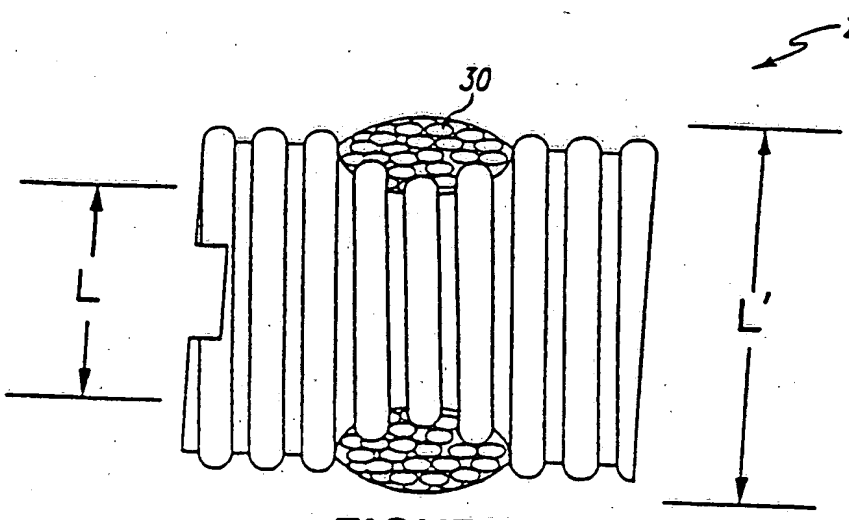

FIGURE 6

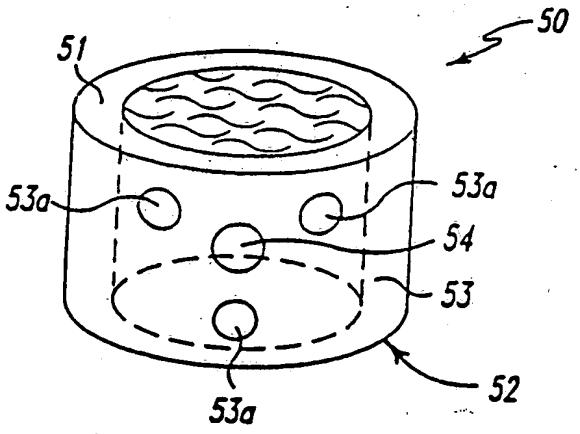

FIGURE 7

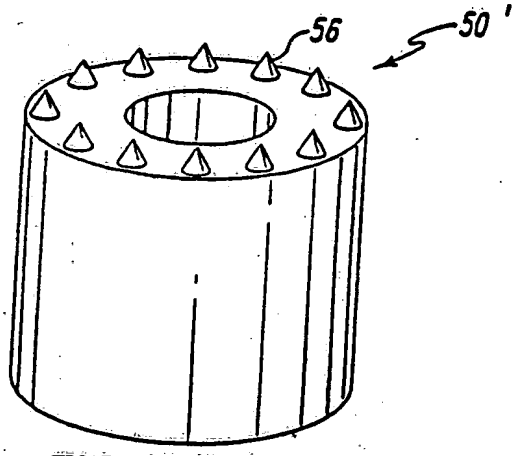

FIGURE 8

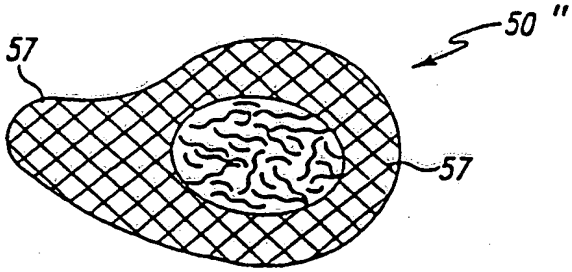

FIGURE 9

INTERNATIONAL SEARCH REPORT

Internati Application No
PCT/US 00/20629

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61L27/36 A61L2/00 //A61L101/20,A61L101/22,A61L101/32, A61L101/34,A61L101/36,A61L101/38

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, PAJ, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	WO 97 27882 A (STERIS CORP) 7 August 1997 (1997-08-07) page 6, line 24 - line 28 page 7, line 2 - line 21 claims --- -/--	1-4, 8-11,33, 34,50,52

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 December 2000	11/12/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Thornton, S

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	US 5 585 116 A (GOLDSCHMIDT PABLO ET AL) 17 December 1996 (1996-12-17) examples 1,4 claims	1-6,8,9, 33,34, 50,52
A	WO 95 32623 A (REGEN BIOLOG INC) 7 December 1995 (1995-12-07) examples 4,5	1-6,9, 21,22, 50,53

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

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Information on patent family members

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